SPONGE MOLECULAR SCREENING FOR ANTIMICROBIAL GENES BY PCR

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ABSTRACT

Molecular biotechnology approach has been applied on sponge for preventing diseases on fishery culture. This is important for anticipating and avoiding the using of amount of sponge in nature. The present study aims to screen the antimicrobial (oxytetracycline and chloramphenicol) genes of sponge. DNA extraction of samples was done using the DNeasy Plant mini kit, Phenol-Chloroform and modification of Phenol-Chloroform methods. The presence of oxytetracycline and chloramphenicol genes in sponge was detected using Polymerase chain reaction (PCR) technique. Result of the study showed that four species (*Sylotella aurantium, Acanthella kletra, Gelliodes fibulatus* and *Auletta* sp. were amplified for oxytetracycline and two species (*Auletta* sp. and *Pericharax* sp.) of sponge were amplified for chloramphenicol at each 226 bp.

KEYWORDS: sponge, chloramphenicol, oxytetracycline

INTRODUCTION

Bacterial disease has been implicated to be one of the most devastating diseases in aquaculture. Due to the impact of outbreak, the production of shrimp and fish has been drastically decreasing not only in pond but also in hatchery. Efforts to prevent and control disease in aquaculture have been carried out by maintaining adequate water quality with low bacterial biomass, sterilizing or filtering recirculated water as well as routine monitoring of shrimp and pond for early diagnosis of problems (Ahmad and Mangampa, 2000). The most common method carried out to overcome the problems was the addition of chemicals, such as formalin and malachite green as well as antibiotics, such as chloramphenicol, oxytetracycline and prefuran (Brown, 1989; Sunarya *et al*., 1996). However the chemicals and antibiotics have been less effective because they give a negative impact on shrimp/fish and also cause resistance on the pathogenic organism. Based on these problems, some researchers have attempted other alternative methods to control the disease.

The rapid growth of knowledge on natural products having various biological activities has provided an alternative application to overcome these problems. The advantages of natural sources are easy to asunder in water and environmentally friendly. Sponge is one member of porifera family that has been reported to contain a biological activity such as antibacteria, antifungi and antibiofouling. BRPBAP has successfully isolated bioactive of sponge for antibacterial such as fenolic acid from *Callyspongia* sp, antifungal such as steroid from *Thionella* sp and antibiofouling such as fenolic acid from *Clathria* sp (Suryati *et al.*, 1997; Muliani *et al.*, 1998; Suryati *et al.*, 1999). In Malaysia, researchers started to use molecular biotechnology approach such as polymerase chain reaction (PCR) method to detect the presence of antimicrobial and cellulase genes in sponge. They found the existence of chloramphenicol gene in *Dysidea* sp. and *Haliclona* sp. and cellulase gene in *Siphonochalina* sp. at 226 and 860 bp respectively (Jaafar *et al*., 2003). In the current work we will report the existence of chloramphenicol gene in *Auletta* sp. and *Pericharax* sp. and oxytetracycline gene in

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Sylotella aurantium, *Acanthella kletra*, *Gelliodes fibulatus* and *Auletta* sp.

The data resulted is expected to be value in formulating more environmentally friendly technique on disease prevention in aquaculture.

MATERIALS AND METHODS

Materials

Sponge materials were collected from Barrang Lompo Island and Awerange Water, Sulawesi Selatan. Primers for identification of the antimicrobial genes were purchased from Qiagent.

Sponge species used are *Aaptos-aaptos*, *Aaptos suberitoides*, *Acanthella* sp., *Acanthella kletra*, *Auletta* sp., *Clathria* sp., *Clathria reinwardhi*, *Callyspongia* sp., *Cribrochalina* sp., *Dysidea* sp., *Gelliodes fibulatus*, *Haliclona* sp., *Jaspis* sp., *Petrosia* sp., *Pericharax* sp., *Pericharax heterorhapis*, *Phokelia flabelata*, *Phylospongia* sp., *Sylotella aurantium, Theonella* sp., *Thalyasias vulpine*, *Xestospongia* sp., *Reniochalina stalagmites* and *Plakortis nigra.*

Methods

Tissue Preservations

Samples was collected from different locations and classified by using coral reef field guide (Barnes, 1982; Allen and Steene, 1994). Prior to the DNA extraction, the samples were washed thoroughly with sterilized sea water, continued with soaking in autoclaved distilled water for three or four times to ensure samples were bacterial-free. All Bacterial-free samples were finely ground by a surgical blade. One hundred mg of sample were directly soaked in 400 mL of buffer AP1 from DNeasy Plant Mini Kit or 500 mL of TNES-Urea for DNA extraction.

DNA Extraction

Extraction of sponge DNA was carried out by using three methods namely; the DNeasy Plant mini kit purchased from Qiagent, Phenol-Chloroform and modification of Phenol-Chloroform methods (Parenrengi *et al*., 2000).

Agarose gel electrophoresis of DNA

A 7.5 mL sample of genomic DNA was mixed with 2.5 mL loading dye, loaded into 0.8% agarose gel and run in 1X TBE buffer (Tris-Boric acid- EDTA) at 50 Volt for 2 hours. The gel was distaining with 0.5 mg/mL ethidium bromida immediately after electrophoresis process is done. Hind III marker was used as a genomic marker. Gels are photographed under a high source of UV transilluminaator (Biometra TI 1) using direct screen instant camera.

PCR amplification

Amplification of the antimicrobial gene was carried out by PCR technique using *Ready to Go* PCR Beads. Primers used to amplify the gene were forward primer (ACAACACCATGCGCCACTGCT) and reverse primer (CGTTCACCACCTTGAGGGACT) for chloramphenicol and forward primer (ACCACTAGGTGAGGTGCAGGA) and reverse primer (TAGTTGCTTAGGCGGTAGGGC) for oxytetracycline genes.

Every PCR reaction mixture contained 3 mL of DNA, 2 mL of each primer and 18 mL of RNase-water Free was prepared for running. The PCR running was set in 30 cycles of 5 min pre start at 94^{o}C, 1 min denaturation at 94^{o}C, 2 min annealing at 64.5^{o}C and 3 min extension at 72^{o}C.

Agarose gel electrophoresis of PCR product

A 7.0 ml sample of PCR product was mixed with 1.5 mL loading dye, loaded onto 2.5% agarose gel, and run as mention above. Gene Ruler 100 bp DNA Ladder Plus was used as standard to identify fragmen of the amplification result of genomic DNA. Based on the documentation gel, genes amplified at 226 bp showed the presence of the antimicrobial (chloramphenicol and oxytetracycline) gene.

RESULTS AND DISCUSSION

Tissue Preservations and DNA extraction

The results indicated that all of the two preservatives used in this study can be used to preserve muscle tissue of sponge species for DNA extraction. Both show to give similar band of genomic DNA. The result of this study exhibit that several genomic DNA of sponge still could not be extracted by DNeasy Plant mini kit and Phenol-Chloroform so that a modification of Phenol-Chloroform methods is needed in their DNA extraction process such as the addition of potassium acetate. The use of chemical materials in this method is

expected to decrease polysachaide content in sponge (Sulandari and Zein. 2003). The smearing background appeared by genomic DNA of the sponge do not show a big influence on amplification process. It indicates the presence of degraded DNA.

PCR amplification of genomic DNA

PCR product of two (*Auletta* sp. and *Pericharax* sp.) and four (*Sylotella aurantium*, *Acanthella kletra*, *Gelliodes fibulatus* and *Auletta* sp.) out of twenty-four sponges showed a single band at 226 bp, which is the expected size of the *clo* gene (Figure 1 and 2) which codes for the antibiotic chloramphenicol and oxytetracyline respectively. The rest of sponges did not show any amplification for the two antibiotics.

The existence of bands at the position of 226 bp is assurance as the expression of the antimicrobial (chloramphenicol and oxytetracycline) gene couples finding their base couples on organism. Previous study on *Auletta* sp. showed this sponge has an activity as bactericide on *Vibrio* sp. (Suryati *et al*., 1995). The result obtained from this study proved that the antimicrobial activity shown by sponge is because of the presence of antimicrobial (chloramphenicol and oxytetracycline) gene, which was amplified at 226 bp. This gene, which expresses in order that sponge has character like antibiotics. The existence of chloramphenicol and oxytetracycline genes in sponge is hoped to be able to change using antibiotics, which have been less effective by transferring to appropriate microbe. However it still needs to be done bioassay test to detect antimicrobial activity of sponge to prove the result of study by molecular.

CONCLUSION

Twenty-four sponge species were screening four antimicrobial genes. Four species (*Sylotella aurantium*, *Acanthella kletra*, *Gelliodes fibulatus* and *Auletta* sp.) were amplified for oxytetracyline and two species of the sponge (*Auletta* sp. and *Pericharax* sp.) were amplified for chloramphenicol genes at 226 bp each. The presence of the two antimicrobial genes is hoped to be able to decrease exploitation of sponge.

REFERENCES

Ahmad, T. and M. Mangampa. 2000. The use of mangrove stands for bioremidation in a close shrimp culture system. In *Proceeding*

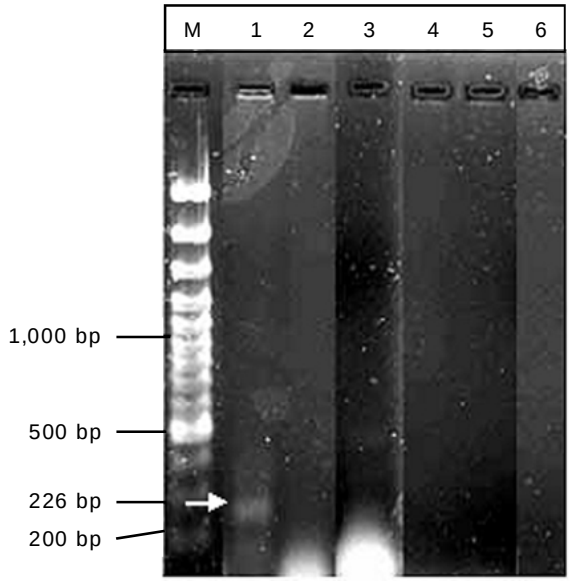

Figure 1. Amplification of the chloramphenicol gene at 226 bp. Lane M = GenRuler 100 bp DNA ladder Plus, Lane 1 (*Auletta* sp.), Lane 2 (*Sylotella aurantium*), Lane 3 (*Pericarax* sp.), Lane 4 (*Acanthella klethra*), Lane 5 (*Gelliodes fibulatus*) and Lane 6 (*Clathria* sp.)

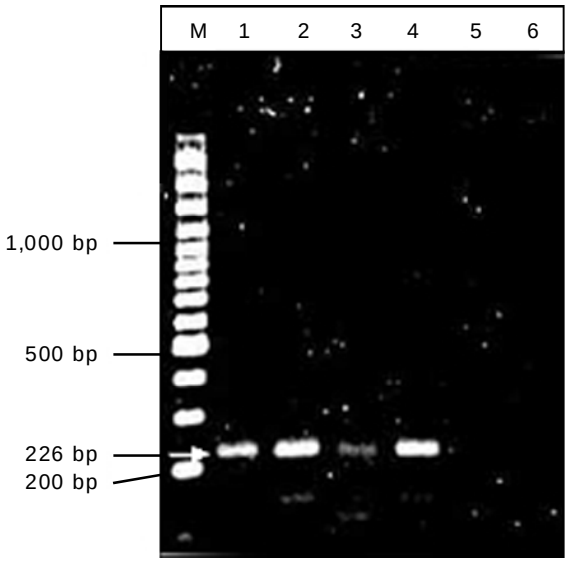

Figure 2. Amplification of the oxytetracycline gene at 226 bp. Lane 1 (*Sylotella aurantium*), Lane 2 (*Acanthella klethra*), Lane 3 (*Gelliodes fibulatus*), and Lane 4 (*Auletta* sp.) amplified the gene whereas Lane 5 (*Callyspongia* sp.) and Lane 6 (*Clathria* sp.) showed no amplification

of International Symposium on marine Biotechnology, ed. L. Hardjito. Jakarta: DFID Press. p. 114-120.

Allen, G.R. and R. Steene. 1994. *Indo-Pacific coral reef*. Field Guide. Tropical Reef Research: Singapore. p. 29-41.

Barnes, R.D. 1982. *Invertebrate Zoology* 4nd. Saunder College Philadelphia. Holt-Saunders Japan Tokyo. p. 41-52.

Brown, J.H. 1989. Antibiotics, their use and abuse in aquaculture. *Aquaculture* 2: 34-43.

Jaafar, F., M.N. Shamsuddin and A. Arshad. 2003. Molecular screening of sponge for antimicrobial and enzyme genes. *To be completed by UPM Proceeding Editors*.

Madeali, M.I., M. Atmomarsono., A. Tompo and Muliani. 1995. Studi kasus penyebab kematian udang windu, Penaeus monodon di tambak intensif. *J. Penelitian Budidaya Pantai.* 9:23-28.

Muliani., E. Suryati. and T. Ahmad. 1998. Isolasi bioaktif sponge untuk fungisida pada benih udang windu (*Penaeus monodon*). *Jurnal Penelitian Perikanan Indonesia.* IV(2): 13-23.

Parenrengi, A., L. Shamsusin., P. Ismail and N.M. Amin. 2000. Preliminary study on DNA level marker of grouper at different buffer preservation and DNA extraction method. *In*: saad, M.S., Faridah, Q.Z., Kadir, M.A., Khalid, M.Z.Z., Mohamad, O., Saleh, G.B. and Panandam, J.M.(Eds.) *Genetic Manipulation*: *Challenges*

Parenrengi, A., E. Suryati., Dalfiah. and Rosmiati. 1999. Studi toksisitas ekstrak sponge *Auletta* sp., *Callyspongia* sp., dan *C. pseudoreticulata* terhadap Nener Bandeng (Chanos-chanos). Jurnal Penelitian Perikanan Indonesia. V(4):15-20.

Sulandari and Zein. 2003. *Panduan praktis laboratorium DNA*. Bidang zoology Pusat Penelitian Biologi Lembaga Ilmu Pengetahuan Indonesia. p. 47-4.

Sunarya, Kukuh, Santosa. and Mufidah. 1996. Study of antibiotic recidue oxytetracyclin on shrimp pond. In *Proceeding of Indonesian fishery simposium*, ed. A. Poernomo. Jakarta: Agriculture Department. p. 295-298.

Suryati, E., Muliani. dan T. Ahmad. 1995. Penapisan bioaktif spons untuk bakterisida dalam bidang perikanan. Prosiding Seminar Nasional Pengelolaan Terumbu Karang Jakarta. p. 164-168.

Suryati, E., Muliani. and T. Ahmad. 1997. Isolasi dan identifikasi bioaktif bunga karang *Callyspongia* sp. yang aktif menghambat pertumbuhan bakteri pada ikan. *Prosiding*

Seminar Nasional Biologi XV. Bandar Lampung 24-26 Juli 1997. p. 29-33.

Suryati, E., A. Parenrengi, and Rosmiati. 1999. Penapisan serta analisis kandungan bioaktif sponge *Clathria* sp. yang efektif sebagai antibiofouling pada teritip (*Balanus amphitrit*). Jurnal Penelitian Perikanan Indonesia. V(3):47-54.